

Summary of the Amendment

The specification has been amended to update the status of the priority and continuing applications. The specification has also been amended to identify the SEQ ID NOs appearing in the figures, and to provide proper SEQ ID NOs for the sequences appearing at page 49, line 39, page 50, line 1, and page 53, line 8. The specification has been amended to correct other informalities and typographical errors. Support for the amendment to the paragraph spanning from page 3, line 31 to page 4, line 6 can be found in original claims 8 and 9 of the application as filed. No new matter has been added.

An abstract has also been provided.

Claims 1 - 37 are canceled without prejudice.

New claims 38 - 44 are added to refer to specific embodiments of the invention. Support for new claims 38 - 44 is found in the original claims, and throughout the specification as filed. No new matter has been added.

New claims 38 - 44 recite prophylactic or therapeutic vaccines comprising CT polypeptides and methods of preparing them.

Conclusion

Applicants respectfully submit that claims 38 - 44 are in condition for allowance. A notice of allowance is earnestly solicited. The Examiner may call the undersigned at 215-557-5901 if a telephonic interview would be helpful.

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Correspondence

All correspondence from the Patent and Trademark Office concerning this application should be sent to:

Alisa A. Harbin, Esq.
Vice President & Associate Chief Patent Counsel
Chiron Corporation
Intellectual Property - R440
P.O. Box 8097
Emeryville, CA 94608-8097

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached pages are captioned "Version with markings to show changes made."

Respectfully submitted,

Date:

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Robin S. Quartin
Registration No. 45,028

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

092157-080201

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Marked up versions of the paragraphs of the specification which are amended by replacement herein.

Paragraph spanning page 3, line 31 to page 4, line 6:

The present invention describes nucleotide and amino acid sequences for three major [H. pylori] *H. pylori* proteins. Specifically, these are the cytotoxin, the "Cytotoxin Associated Immunodominant" (CAI) antigen, and the heat shock protein. None of the complete amino acid sequences for these proteins has been known, nor have their genes been identified. The present invention pertains to not only these purified proteins and their genes, but also recombinant materials associated therewith, such as vectors and host cells. The present invention provides cytotoxin polypeptides that exhibit substantially no toxicity, or substantially reduced toxicity. The present invention also provides CAI and heat shock polypeptides that exhibit no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity. The understanding at the molecular level of the nature and the role of these proteins and the availability of recombinant production has important implications for the development of new diagnostic for [H. pylori] *H. pylori* and for the design of vaccines that may prevent [H. pylori] *H. pylori* infection and treat disease.

Brief Description of the Drawings section at page 4:

[Fig. 1 is] Figs. 1A, 1B, and 1C (SEQ ID NO:2) comprise the nucleotide sequence for the cytotoxin (CT) protein.

Fig. 2 (SEQ ID NO:3) is the amino acid sequence for the cytotoxin (CT) protein.

Fig. 3 is a map of the [cai] *cai* gene for the CAI protein and summary of the clones used to identify and sequence this gene.

[Fig. 4 is] Figs. 4A through 4F (SEQ ID NO:4 and SEQ ID NO:5) comprise the nucleotide and amino acid sequences of the CAI antigen. The numbers along the left-hand margins of Figs. 4A, 4C, and 4E designate the amino acid positions, and the numbers along the right-hand margins of Figs. 4B, 4D, and 4F designate the nucleotide positions.

[Fig. 5 is] Figs. 5A, 5B, and 5C (SEQ ID NO:7 and SEQ ID NO:6) comprise the nucleotide and amino acid sequences of the heat shock protein (hsp).

Paragraph spanning page 49, line 30 to page 50, line 9:

DNA manipulation was performed using standard procedures. DNA sequencing was performed using Sequenase 2.0 (USB) and the DNA fragments shown in Fig. 3 subcloned in [Bluscript] Bluescript KS+. Each strand was sequenced at least three times. The region between nucleotides 1533 and 2289, for which a DNA clone was not available, was amplified by PCR and sequenced using asymmetric PCR, and direct sequencing of amplified products. The overlapping of this region, was confirmed by one and double side anchored PCR: an external universal anchor (5'-GCAAGCTTATCGATGTCGACTCGAGCT-3' (SEQ ID NO:1) / 5'-GACTCGAGTCGACATCGA-3' (SEQ ID NO:8)) containing a protruding 5' HindIII sequence, and the recognition sites of ClaI, SalI, XhoI, was ligated to primer-extended DNA and amplified. A second round of PCR using nested primers was then used to obtain fragments of DNA suitable for cloning and sequencing. DNA sequence data were assembled and analyzed with the GCG package (Genetics Computer Group, Inc., Madison, WI) running on a VAX 3900 under VMS. The GenBank and EMBL databases were examined using the EMBL VAXcluster.

Paragraph spanning page 52, line 15 to page 53, line 9:

The [cai] cai gene coded for a putative protein of 1147 amino acids, with predicted molecular weight of 128012.73 Daltons and an isoelectric point of 9.72. The basic properties of the

purified protein were confirmed by two dimensional gel electrophoresis. The codon usage and the GC content (37%) of the gene were similar to that described for other [*H. pylori*] *H. pylori* genes (13, 26). A putative ribosome binding site: AGGAG, was identified 5 base pairs upstream from the proposed ATG starting codon. Computer search for promoter sequences of the region upstream from the ATG start codon, identified sequences resembling either -10 or -35 regions, however, a region with good consensus to an [*E. coli*] *E. coli* promoter, or resembling published [*H. pylori*] *H. pylori* promoter sequences was not found. Primer extension analysis of purified [*H. pylori*] *H. pylori* RNA showed that 104 and 214 base pairs upstream from the ATG start codon there are two transcriptional starts sites. Canonical promoters could not be identified upstream from either transcriptional [*E. coli*] *E. coli* is also recognizing a promoter in this region, however, it is not clear whether [*E. coli*] *E. coli* recognizes the same promoters of [*H. pylori*] *H. pylori* or whether the [*H. pylori*] *H. pylori* DNA that is rich in A-T provides [*E. coli*] *E. coli* with regions that may act as promoters. A rho independent terminator was identified downstream from the stop codon. In Fig. 4, the AGGAG ribosome binding site and terminator are underlined, and the repeated sequence and motif containing 6 asparagines are boxed. The CAI antigen was very hydrophilic, and did not show obvious leader peptide or transmembrane sequences. The most hydrophilic region was from amino acids 600 to 900, where also a number of unusual features can be observed: the repetition of the sequences EFKNGKNKDFSK (SEQ ID NO:9) and EPYIA (SEQ ID NO:10), and the presence of a stretch of six contiguous asparagines (boxed in Fig. 4).

Paragraph at page 60, spanning lines 15 to 29:

The purified fusion protein was tested by Western blot using sera of patients infected by [*H. pylori*] *H. pylori* and affected by atrophic and superficial gastritis, and patients with duodenal and gastric ulcers: most of the sera recognized the recombinant protein. However, the degree of recognition greatly varied between different individuals and the antibody levels did not show any

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obvious correlation with the type of disease. In addition, antibodies against [H. pylori] H. pylori antigens and in particular against hsp protein were found in most of the 12 sera of patients affected by gastric carcinoma that were tested. Although [H. pylori] H. pylori hsp recognition could not be put in relation with a particular clinical state of the disease given the high conservation between [H. pylori] H. pylori hsp and its human homolog, it is possible that this protein may induce autoimmune antibodies cross-reacting with the human counterpart. This class of homologous proteins has been implicated in the induction of autoimmune disorders in different systems. [Thenpresent] The presence of high titers of anti-[H. pylor] H. pylori hsp antibodies, potentially cross-reacting with the human homolog in dispeptic patients, suggests that this protein has a role in gastroduodenal disease. This autoreactivity could play a role in the tissue damage that occurs in [H. pylori] H. pylori-induced gastritis, thus increasing the pathogenic mechanisms involved in the infection of this bacterium.

In the claims:

Claims 38 - 44 have been added.

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